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# Relationships between antral follicle count, blood serum concentration of anti-Müllerian hormone and fertility in mares

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## Summary

The anti-Müllerian hormone (AMH) plays an inhibitory role during folliculogenesis by regulating the number of follicles entering the growing pool. Antral follicle counts (AFC) are highly correlated with serum AMH concentrations and both appear to be related to the ovarian reserve in several species. Few data on AMH and AFC in mares exist, especially with regard to fertility. Therefore, the objective of the current study was to investigate the interrelationship between antral follicle count, serum AMH concentrations and fertility outcome in mares. One hundred and twenty-seven mares were enrolled in the study and grouped according to their reproductive status. Around time of estrus, serum AMH concentrations and AFC before and after ovulation were determined. Mares were artificially inseminated and pregnancy diagnosis was performed 14 to 18 days later. A high inter-individual variability in AFC and AMH concentration and a positive relationship between AMH and AFC for follicles ≤ 30 mm in diameter were observed, with a stronger correlation in mares older than 18 years. A high correlation between AFC measurements before and after ovulation was identified. The AFC after ovulation was higher than AFC before ovulation. AMH concentrations were neither related to the mares' reproductive status nor to age, number of cycles needed for pregnancy and pregnancy outcome. Excepted for a higher AFC in the middle-aged mares (9-18 years) compared to the younger mares (< 9 years), no associations between AFC and age, reproductive status as well as fertility of mares were found. This study confirms the relationship between AFC and AMH and a high degree of reproducibility of AFC measurements. However, based on our findings, neither AFC nor AMH are useful predictors of fertility in mares.

**Key words:** AMH, equine, follicle count, ovary, reproductive status, ultrasonography

## Zusammenhänge zwischen der Anzahl antraler Follikel, der Serumkonzentration von Anti-Müller-Hormon und der Fertilität von Stuten

Das Anti-Müller-Hormon (AMH) hemmt während der Follikulogenese die Anzahl heranwachsender Follikel. Die Anzahl der antralen Follikel («antral follicle count», AFC) korreliert stark positiv mit der Serumkonzentration von AMH und beide sind bei mehreren Spezies ein Indikator für die ovarielle Reserve. Da bisher wenige Daten über AFC und AMH bei Stuten, insbesondere im Hinblick auf deren Auswirkungen auf die Fertilität vorliegen, sollte dies in der vorliegenden Arbeit untersucht werden. Hundertsiebenundzwanzig Stuten wurden in die Studie eingeschlossen und nach Reproduktionsstatus gruppiert. Die AFC und die AMH-Konzentration wurden während der Rosse vor und nach der Ovulation ermittelt. Die Stuten wurden künstlich besamt und die 14 bis 18 Tage später eine Trächtigkeitsunteruchung durchgeführt. Es bestanden hohe Variabilität im AFC und in der AMH-Konzentration zwischen den Stuten und ein positiver Zusammenhang zwischen AMH und AFC für Follikel mit einem Durchmesser ≤ 30 mm, wobei die Korrelation bei Stuten > 18 Jahre stärker ausgeprägt war. Die AFC-Messungen vor und nach Ovulation korrelierten sehr gut miteinander, wobei der AFC nach Ovulation grösser war als vor der Ovulation. Die AMH-Konzentrationen waren unabhängig vom Alter, dem Reproduktionsstatus, der Anzahl benötigter Zyklen bis zur Erreichung einer Trächtigkeit und dem Trächtigkeitsausgang. Mit Ausnahme der Beobachtung, dass die AFC bei mittelalten Stuten (9–18 Jahre) höher als diejenige bei AFC jüngerer Stuten (< 9 Jahre) war, konnten keine Assoziationen zwischen AFC und Alter, Reproduktionsstatus oder Fertilität erkannt werden. Diese Studie bestätigt den Zusammenhang zwischen AFC und AMH und eine hohe Repro-

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This work is dedicated to Prof. Adrian Steiner 60th birthday.

duzierbarkeit der AFC-Messungen. Allerdings können anhand der vorliegenden Ergebnisse weder AFC noch AMH als brauchbare Prädiktoren für die Fruchtbarkeit von Stuten herangezogen werden.

**Schlüsselwörter:** AMH, Pferd, Anzahl Follikel, Ovar, Reproduktionsstatus, Ultraschall

## Introduction

The number of follicles present in female ovaries is established during the fetal period and, due to apoptotic processes resulting in atresia, it already starts to decrease before birth.[1,2] The size of the pool of non-growing follicles (NGF), which includes primordial, intermediate and small primary follicles, varies greatly between individuals and species and is also age-dependent.[2–9] The total number of resting follicles at birth averages 520'000 in humans and 150'000 in bovines.[3,8] Estimates for the number of oocytes present in the ovaries of the mare at birth are not available. In adulthood, numbers vary between 0 and 800'000 (average 130'000) in women, between 3'000 and 150'000 in cows and between 5'600 and 75'000 in mares.[3,4,8]

The ovarian reserve is defined as the number of follicles left in the ovaries at a given time and can only be accurately assessed *post mortem* since follicles cannot be measured by clinical methods before having reached the antral stage.[9–12] Antral follicles are fluid-filled with a diameter ≥ 2 mm and can therefore be visualized by transvaginal (women, cows) or transrectal (cows, mares) ultrasonography.[13–18] Their number is directly related to the number of primordial follicles, and the antral follicle count (AFC) is highly repeatable within individuals and widely used as quantitative marker of the ovarian reserve.[17–21]

The anti-Müllerian hormone (AMH) is a glycoprotein expressed in granulosa cells of primary, pre- and early antral follicles and appears to regulate the number of follicles that enter the growing follicle pool, playing a major regulatory role in female reproduction during folliculogenesis.[22–27] Serum AMH is a good indicator of the follicular activity and its concentration has been shown to be stable within and across cycles.[12,28–30] Moreover, AMH is positively correlated to AFC in several mammalian species including mares and both are used as indicators of fertility, although their relationship to the latter couldn't always be demonstrated.[15,17–21,29,31–38] In cows and goats, both biomarkers are predictive of superovulatory response, and AMH is widely used in women to predict *in vitro* fertilization and pregnancy outcomes.[16,29,39–42]

Fertility, AMH concentrations and AFC decline with age in all the aforementioned species, but there is a great variability of both AMH concentration and AFC even between individuals of the same age and therefore the onset of reproductive senescence associated with reduced ovarian reserve varies considerably.[8,12,15,18,21,30,43–47] In some studies, no significant correlations between AMH and/or AFC and pregnancy rate have been reported in mares and women, indicating that besides the number of primordial follicles present in the ovaries and the AFC and AMH correlated to it, other factors such as oocyte quality are likely to play a role.[33–35] On the other hand, the study by Ball et al. (2019)[12] demonstrated a relationship between AMH and fertility, which was reduced in mares with low serum AMH.

To our knowledge, no studies on the effect of both AFC and AMH concentrations on mare fertility have been published. Therefore, the objective of the current study was to investigate the interrelationship between antral follicle count, serum AMH concentrations and fertility outcome in mares.

## Materials and Methods

### Mares

One hundred and twenty-seven Warmblood mares presented for artificial insemination (AI) at a private Center of Equine Reproduction in Switzerland during the 2014 breeding season were enrolled in the study. Clinically healthy mares, with an estrous cycle length of 18 to 23 days and not bred until study start were included in the study and grouped according to their reproductive status: maiden mares (MM), lactating mares with a foal (LM), barren mares inseminated during the preceding season (BILS) and barren mares not inseminated during the preceding season (BNILS). Mares were defined as barren if they hadn't been pregnant during the last one or two breeding seasons. Thus, the BNILS group also comprised mares that weren't bred for reasons other than health or fertility, such as financial costs and sporting activity. In barren mares, cytological and bacteriological examination of uterine swab was performed at enrollment, and mares with positive results were excluded from the study. Mares were kept in straw-bedded

boxes with permanent access to hay, grain and water, and were turned out daily to pasture.

Breeding management

Around time of estrus, the mares were examined one to three times daily by transrectal palpation and ultrasonography with a 7.5 MHz linear probe (MyLab 30, Esaote, Köln, Germany) to assess cycle stage and day of ovulation. In the presence of distinct estrus signs (dominant follicle ≥ 35 mm, soft consistency, dilation of the cervix, endometrial oedema characterized by the detection of endometrial folds on cross-sectional ultrasound images), ovulation was induced with 3,000 IU of hCG (Chorulon 5,000 ad us. vet., Veterinaria SA Freienbach, Pfäffikon, Switzerland). Venous blood samples of the 127 mares were obtained for the analysis of anti-Müllerian hormone (AMH) concentration. Blood collection in a serum clot activator tube was performed passively through the inserted needle shortly prior to hCG injection.

Following injection, mares were examined every 6 to 12 hours, depending on the appearance of the dominant follicle and the evolution of uterine oedema. Artificial insemination occurred when the dominant follicle(s) showed a change in shape with loss of spherical appearance and a double wall on the ultrasound images,[48,49] or if ovulation was observed. Ovulation was verified in the 12 hours after AI and, if a mare didn't ovulate, re-examinations were scheduled every 6h until ovulation occurred.

All mares were bred by AI performed deeply into the uterine horn ipsilateral to the ovulatory follicle (cryopreserved semen) or into the uterine body (fresh semen).

Presence of intrauterine fluid was assessed in the 12 hours following AI and was treated according to a predefined protocol, consisting of the use of 10 IU oxytocin i.m. (Intertocin-S ad us. vet., MSD, Luzern, Switzerland) if fluid accumulation was < 2 cm and of uterine flushing with 3 to 6 liter 0.9% NaCl followed by 10 IU oxytocin administered intravenously if accumulation was ≥ 2 cm. Treated mares were examined 12 to 24 hours later and, if needed, the treatment was repeated.

Pregnancy was diagnosed by ultrasonography 14 to 18 days after ovulation. In case of a negative outcome, mares were re-inseminated in the following cycle. Semen of other, more fertile stallions – selected according to the pregnancy rate achieved by AI in other mares – was used for re-insemination in 15 mares during the remaining breeding period.

Fertility of the mares was estimated by calculating the first cycle pregnancy rate (FCPR), the seasonal pregnancy rate (SPR) and the number of cycles needed per pregnancy (NCP).

## Determination of antral follicle count (AFC)

The number of antral follicles present during heat before ovulation was determined by transrectal ultrasonography at the time of hCG injection in 97 of the 127 mares. One of two people (HA and BK) examined the mares and made videos of the ovaries in sonographic 2D-mode (MyLab 30, Esaote, Köln, Germany), recording sequences displaying the view from the hilus to the ovulation fossa for about 8 seconds. The procedure was repeated after ovulation on 58 of the 97 mares to investigate if the presence of pre-ovulatory follicles affected the antral follicle count. All vid-

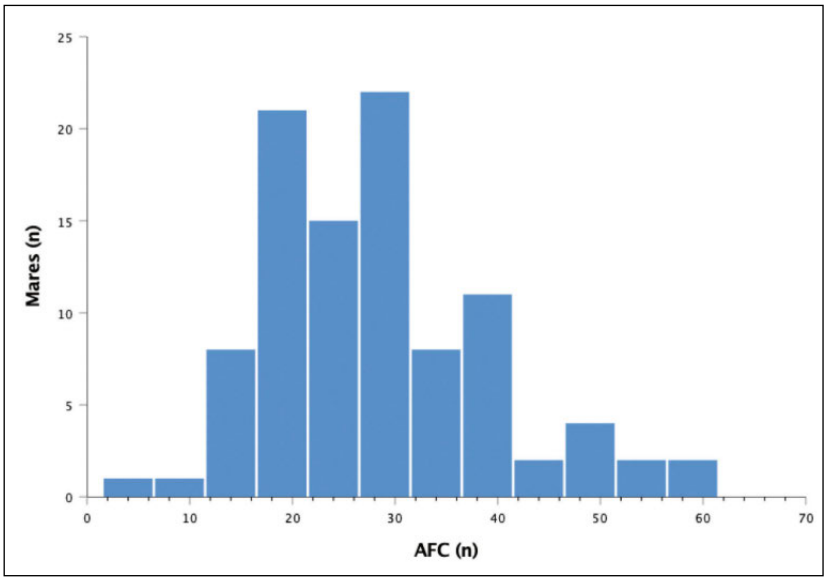


**Figure 1:** Frequency distribution of the antral follicle count (AFC) in 97 mares.

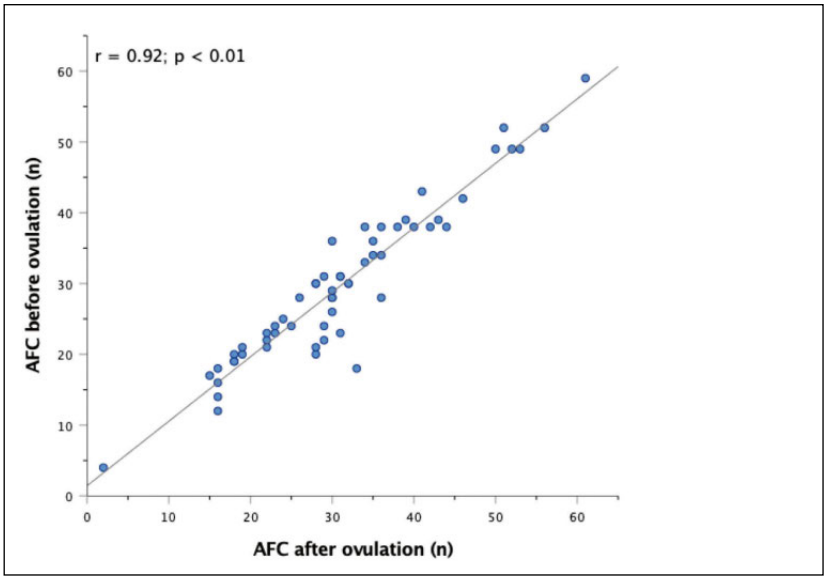


**Figure 2:** Relationship between the antral follicle count (AFC) determined before and after ovulation in 58 mares.

eos were processed separately by one person (HA) and evaluated as described by Claes et al. (2015).[18] each follicle with a diameter ≥ 2 mm was counted, its largest diameter measured and classified into a group according to its diameter (2-5, 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, 35-40, > 40 mm).[50] To assess the accuracy of the follicular counting, multiple videos per ovary were made in 20 mares at the beginning of the study.

### Determination of serum AMH concentration

Blood samples were centrifuged (1200 × g, 10 min) immediately after collection and 0.5 mL serum were frozen (–19 °C) until analysis. Measurements were performed with the commercially available ELISA kit AMH Gen II A79765 from Beckman Coulter (Brea, California, USA) according to the instructions of the manufacturer. The intra- and inter-assay coefficients of variation for the AMH assay were 4.5% and 5.5%, respectively. The limit of sensitivity of the assay was 0.17 ng/mL.

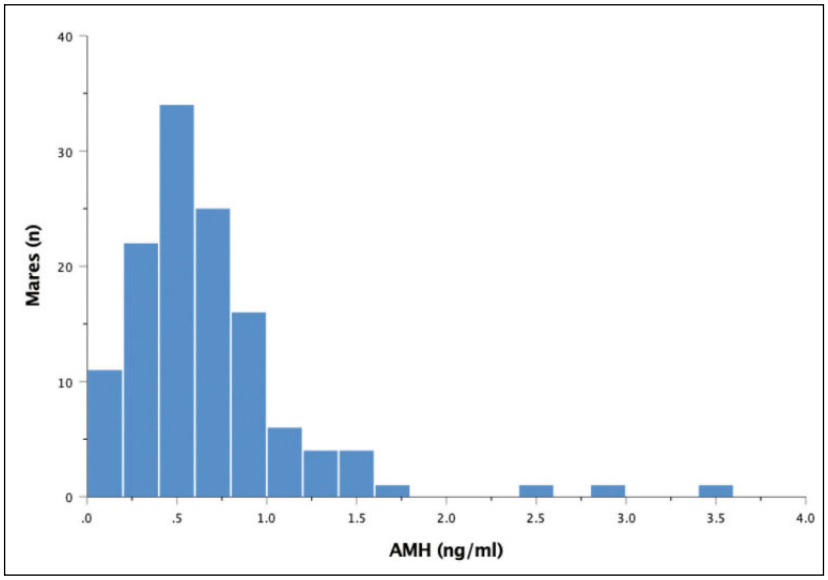


**Figure 3:** Serum concentration of Anti-Müllerian hormone (AMH) in 127 mares.

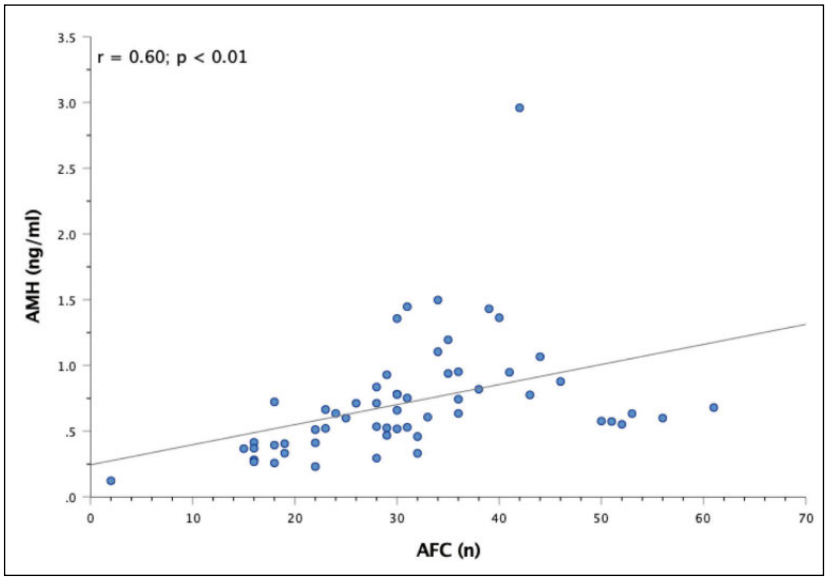


**Figure 4:** Relationship between the antral follicle count (AFC) of follicles with a diameter ≤30 mm and the serum concentration of anti-Müllerian hormone (AMH) in 97 mares.

### Statistical analysis

Statistical analyses were conducted using the software StatView 5.0 (SAS Institute Inc., Cary, NC, USA) and SPSS Version 25 (SPSS Inc., Chicago, IL, USA). Data were presented as mean ± SD or median, minimum and maximum values as well as box plots showing median values, 25%, and 75% quartiles, 1.5 IQR and outliers. Data distribution for normality was tested visually and by means of the Shapiro-Wilk test. Data were also stratified according to quartiles into lower (25%), mid-50% (second and third quartiles combined - 50%) and upper (25%) quartiles for AFC and serum AMH concentration and age was divided into 3 groups: young (3–8 years), middle-aged (9–18 years) and old mares (≥ 19 years). Correlations between AFC before and after ovulation and between AFC and AMH concentrations were determined using the nonparametric Spearman's rank correlation. Differences between AFC before and after ovulation were analyzed using the Wilcoxon signed-rank test. Differences in AFC and serum AMH between different age groups, reproductive status category, fertility outcome, presence of intrauterine fluid after AI and number of inseminations were determined using the Mann-Whitney-U test or the Kruskal-Wallis test followed by post-hoc tests using the Bonferroni correction for multiple comparisons. The Chi-square test was used to identify associations between reproductive status, age group, AFC groups, AMH groups, presence of intrauterine fluid after AI and fertility outcome. Differences were considered significant at $p \leq 0.05$.

## Results

### Age, reproductive status, breeding and fertility

The mean age of the mares was 12.9 ± 4.5 yrs (range: 3 to 23 yrs). Twenty-three mares belonged to the "young" group (= 18.1%), 92 to the "middle-aged" (= 72.4%) and 12 to the "old" group (= 9.4%). Of the 127 mares, 22 (17.3%) were MM, 51 (40.1%) were LM, 27 (21.3%) were BNILS and 27 (21.3%) were BILS. Fifty-three mares got pregnant after the first insemination (FCPR = 41.7%) and at the end of the season, 94 mares were pregnant and 33 mares were not (SPR = 74.0%). Fresh semen was used on 19 mares (14.9%), cryopreserved semen on 108 mares (85.1%). Mares were inseminated with 79 different stallions 1.76 ± 0.96 times (min. 1, max. 5) and 1.2 ± 1.02 cycles per pregnancy were needed during the breeding season.

**Table 1:** Number of mares (n) having at least one antral follicle in the respective size category (diameter) and preovulatory antral follicle counts (median, minimum, maximum) according to the follicle size categorized by their diameter.

| Diameter (mm) | 2-5 | 6-10 | 11-15 | 16-20 | 21-25 | 26-30 | 31-35 | 36-40 | >40 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Mares (n) | 97 | 97 | 97 | 97 | 97 | 97 | 97 | 97 | 97 | 97 |
| Median | 9 | 6 | 5 | 2 | 1 | 0 | 0 | 1 | 0 | 28 |
| Minimum | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Maximum | 37 | 21 | 17 | 7 | 5 | 2 | 2 | 2 | 2 | 59 |

Delayed uterine clearance was observed in 54 mares (11 of which were maiden mares), and intrauterine flushing was performed in 15 mares (11.8%), whereas oxytocin was injected in 39 mares (30.7%). The proportion of pregnant mares at the end of the breeding season didn't differ across categories of reproductive status, presence of intrauterine fluid and type of semen used ($\chi^2 = 0.59$, $p > 0.05$; $\chi^2 = 4.01$, $p > 0.05$ and $\chi^2 = 0.001$, $p > 0.05$; respectively).

## Antral follicle count

There was a high variability in the AFC between mares (Fig. 1). A very high correlation could be observed between the AFC measurements before and after ovulation (Fig. 2) ($rs = 0.92$; $p < 0.01$), but the AFC on both ovaries was lower ($Z = -2.42$, $p \leq 0.05$) before ovulation (median 28; min. 4; max. 59) than after ovulation (median 30; min 2; max 61). The AFC within the different follicular classes decreased with increasing diameter of the follicles up to 25 mm diameter (Tab. 1).

## Serum anti-Müllerian hormone concentration

The AMH concentrations showed a high variability between mares (Fig. 3). It ranged from 0.07 to 3.56 ng/mL with a median value of 0.59 ng/mL and three outliers (2.49, 2.96 and 3.56 ng/mL).

Serum AMH concentration and AFC were positively correlated for smaller follicles up to 30 mm of diameter (Fig. 4; $r_s = 0.60$, $p < 0.01$). The degree of correlation differed between mares of different groups of age (Fig. 5). It was strong in old mares ($r_s = 0.90$, $p < 0.01$) and moderate in middle-aged ($r_s = 0.60$, $p < 0.01$) and young mares ($r_s = 0.57$, $p \leq 0.05$).

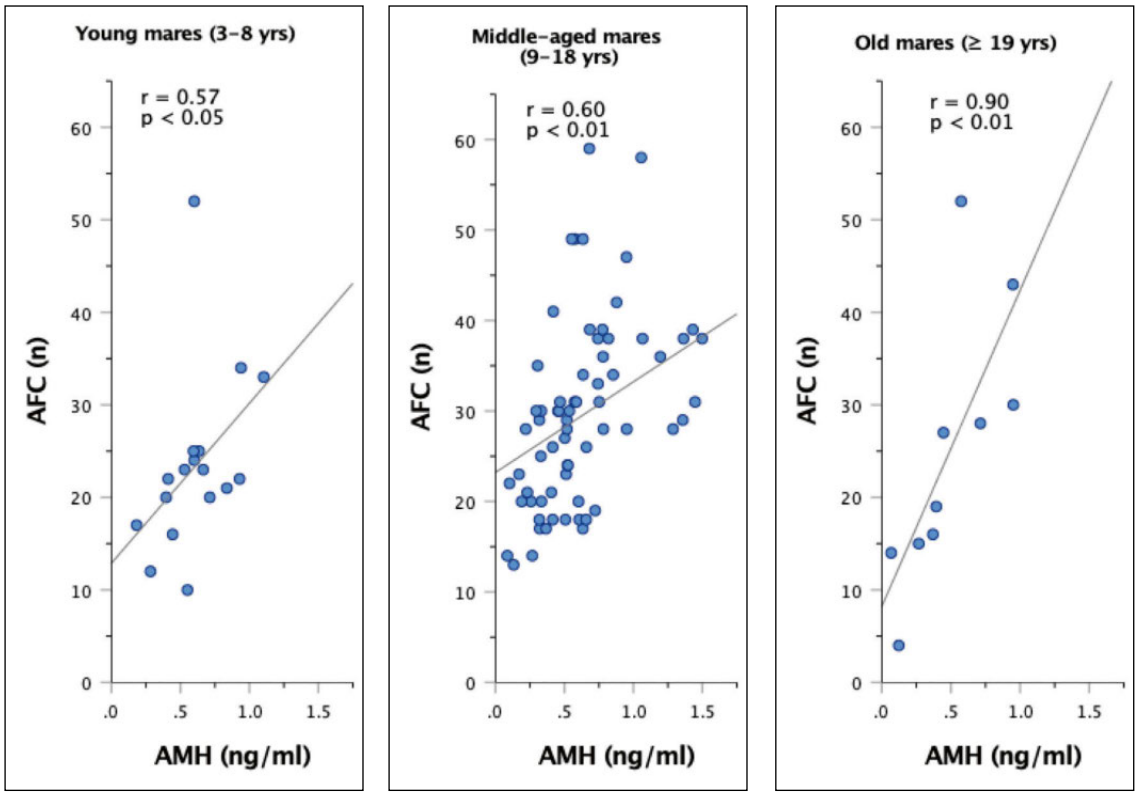


**Figure 5:** Relationship between the serum concentration of anti-Müllerian hormone (AMH) and the antral follicle count (AFC) in 97 mares of different age groups (young (3–8 years; n = 23), middle-aged (9–18 years; n = 92) and old mares (≥19 years; n = 12).

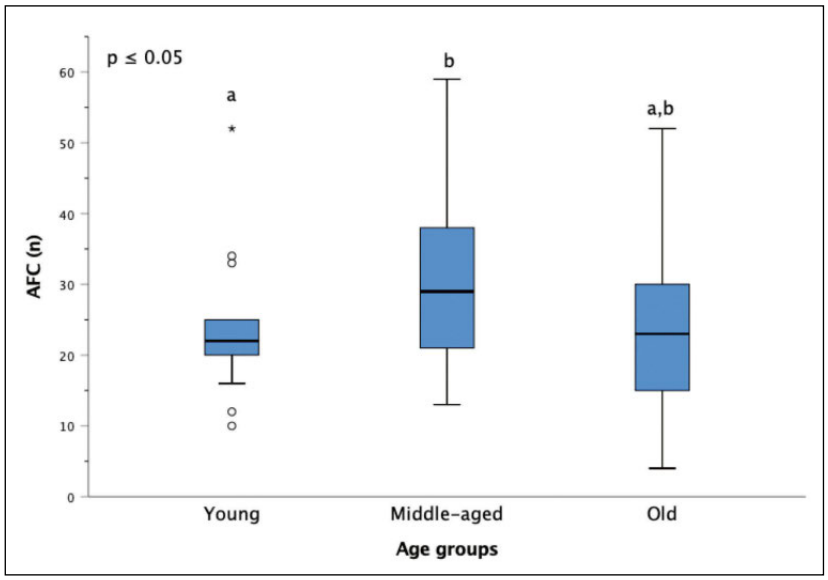


**Figure 6:** Antral follicle count (AFC) in young (3–8 years; n = 92 years) and old mares (≥ 19 years; n = 12). The boxplots present median values, 25% and 75% quartiles, 1.5 interquartile range (IQR) and outliers, indicated by individual dots and an asterisk (representing an extreme value). Different letters above the box plots indicate statistically significant differences.

### Relationships between antral follicle count, serum concentration of anti-Müllerian hormone and age, reproductive status, fertility in mares

Middle-aged mares (9-18 years) had higher AFC ($\chi^2$ = 6.93, p ≤ 0.05) than young mares (3-8 years), but the difference between old mares (≥ 19 years) and the other age groups was not significant (Fig. 6). No relationships (p > 0.05) between AFC and reproductive status, presence of intrauterine fluid after AI, number of cycles needed for pregnancy and pregnancy outcome could be identified (Fig. 7-10). The AMH concentrations were neither related (p > 0.05) to the mares' reproductive status, nor to age, presence of intrauterine fluid after AI, pregnancy, or number of cycles needed for pregnancy (Fig. 7-10).

## Discussion

The observed values and the strong inter-individual variability in AFC and AMH concentration in mares are in line with published findings.[12,18,30,36,43] Similar variations were described for cows and women.[17,45–47]

The high positive correlation between AFC determined before and after ovulation indicates a high degree of reproducibility of the measurements by using two-dimensional B-mode sonography. The result that AFC was higher after ovulation than before ovulation might appear counterintuitive, but could be justified by technical and anatomical explanations. The presence of a round or oval structure like a dominant follicle can produce sonographic artifacts such as acoustic or edge shadows and lead to a loss of focus due to its size, thus masking the presence of other follicles.[51] Furthermore, the migration of the large preovulatory follicle towards the ovulation fossa, which is distant from the ultrasound

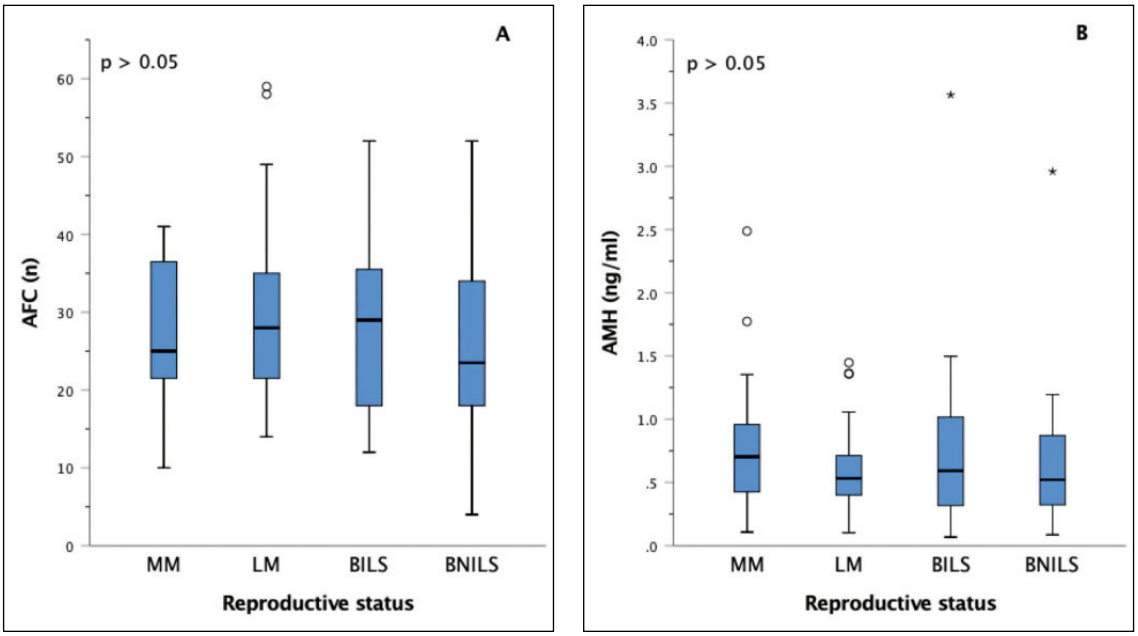


**Figure 7:** Antral follicle count (AFC; Fig. A) and anti-Müllerian hormone concentration (AMH; Fig. B) in maiden (MM; n = 22), lactating mares (LM; n = 51) and in barren mares not inseminated during the last season (BNILS; n = 27) or inseminated during the last season (BILS; n = 27). The boxplots present median values, 25% and 75% quartiles, 1.5 interquartile range (IQR) and outliers, indicated by individual dots and asterisks (representing extreme values).

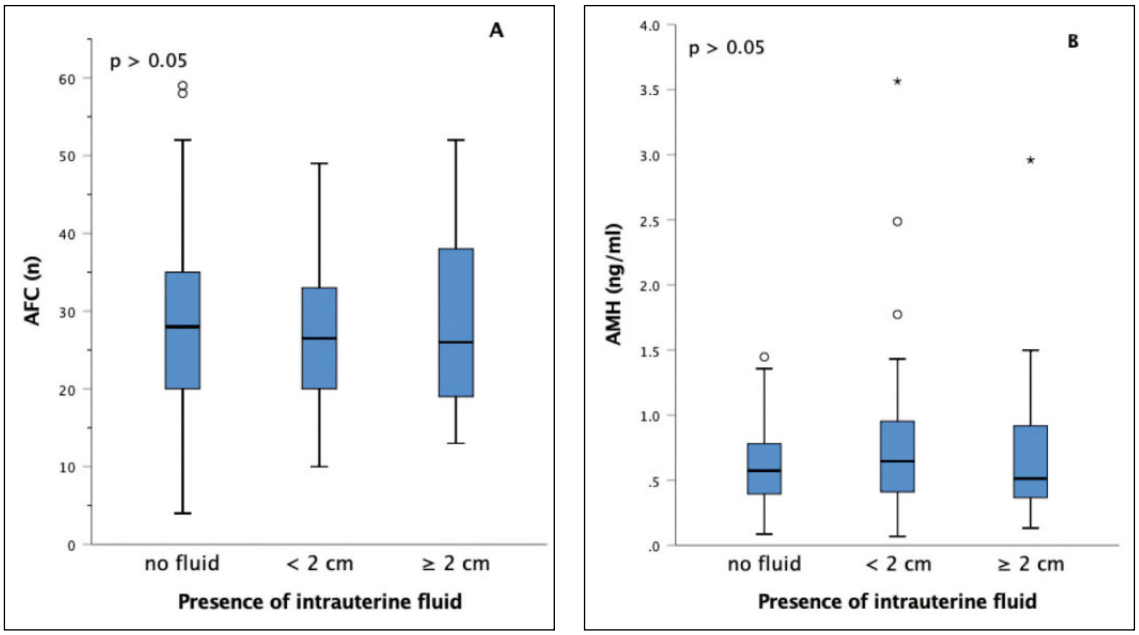


**Figure 8**: Antral follicle count (AFC; Fig. A) and anti-Müllerian hormone concentration (AMH; Fig. B) in mares without (n = 73) or with (< 2 cm, n = 39; ≥ 2 cm, n = 15) intrauterine fluid accumulation. The boxplots present median values, 25% and 75% quartiles, 1.5 interquartile range (IQR) and outliers, indicated by individual dots and asterisks (representing extreme values).

probe, might also have a negative impact on the quality of the ultrasonographic images and lead to an underestimation of the AFC.[52] According to our results, the accuracy of the determination of AFC is higher in ovaries without large follicles.

Another observation was that AFC decreased with increasing size of the follicles present on the evaluated ovary and was higher when follicles didn't exceed 25 mm of diameter. This finding is not surprising, since growth of smaller follicles is inhibited by AMH produced by the antral follicles until follicular deviation,

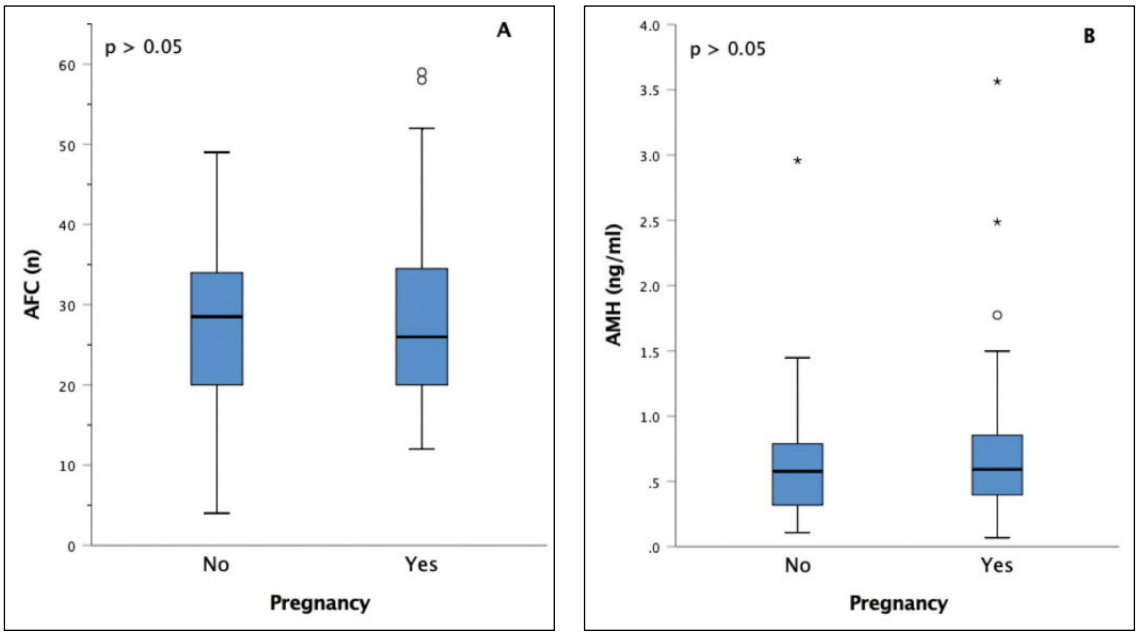


**Figure 9**: Antral follicle count (AFC; Fig. A) and anti-Müllerian hormone concentration (AMH; Fig. B) in mares which got pregnant (n = 94) or not (n = 33) during the breeding season. The boxplots present median values, 25% and 75% quartiles, 1.5 interquartile range (IQR) and outliers, indicated by individual dots and asterisks (representing extreme values).

which occurs at a (dominant) follicle diameter of approximatively 22 mm.[53,54] This concept also explains the positive correlation between AMH concentration and AFC for follicles ≤ 30 mm, although in the study of Claes et al. (2015)[18] this phenomenon was observed only with follicles ≤ 20 mm. Similar dynamics and correlations have been identified in several other mammal species.[19–21,29,32]

Middle-aged mares (9-18 years) had significantly higher AFC than young mares (3-8 years), but there was no difference between the AFC in older mares and the other age groups. Moreover, the correlation between AMH and AFC was influenced by age. Associations between these two biomarkers and age have been demonstrated in mares, cows and women, and are likely to reflect the follicular origin of AMH and the diminishing ovarian reserve.[8,15,18,21,43,44] Similarly to our findings, Claes et al. (2015)[18] observed a strong or moderate relationship between AFC and AMH concentrations in older resp. middle-aged mares, but, in contrast to our study, didn't find a correlation in young mares. In the same study, AFC was significantly lower in older mares, which was explained by its correlation to the number and the age-related depletion of primordial follicles (proven by the same authors in a study published in 2017[9]). Furthermore, others reported that AMH concentrations begin to decline in mares only after 20 years of age.[43] In contrast, in our study older mares didn't have significantly lower AMH and AFC measurements compared to other age groups, but the AFC was higher in middle-aged mares than in young mares. This could be explained by the relatively unequal distribution of mares between the different age groups in our study, but also by the previously reported large individual differences in follicular populations between individuals of the same age.[12,18,30,43,45–47] In other species, other factors are known to affect AMH and/or AFC: in cattle and sheep, studies showed that maternal undernutrition during the first trimester has a negative impact on the AFC in the offspring.[55–58] Moreover, a study found that heifers from dams affected by chronic mastitis (clinical and subclinical) had lower AMH concentrations than their peers issued from healthy cows.[59] In studies performed *in vivo* (murine ovaries) and *ex vivo* (bovine ovaries), Bromfield and Sheldon (2013)[60] could demonstrate that *E. coli* lipopolysaccharide (LPS) decreases the primordial follicle pool. Genetic factors could also possibly affect AFC and AMH. Differences between breeds have been demonstrated for AFC and AMH in cows and a study in mares showed that Standardbred mares had significantly higher mean AMH concentrations than Thoroughbred mares.[34,61,62]

In the current study, AMH concentrations and AFC were neither related to the number of cycles needed for pregnancy nor to the pregnancy outcome at the end of the season. As previously stated, a relationship between AMH and fertility has been described in mares, cows and women. Ball et al. (2019)[12] found that fertility was decreased in mares in the lowest AMH quartile. Both AFC and AMH are predictive of superovulatory re-

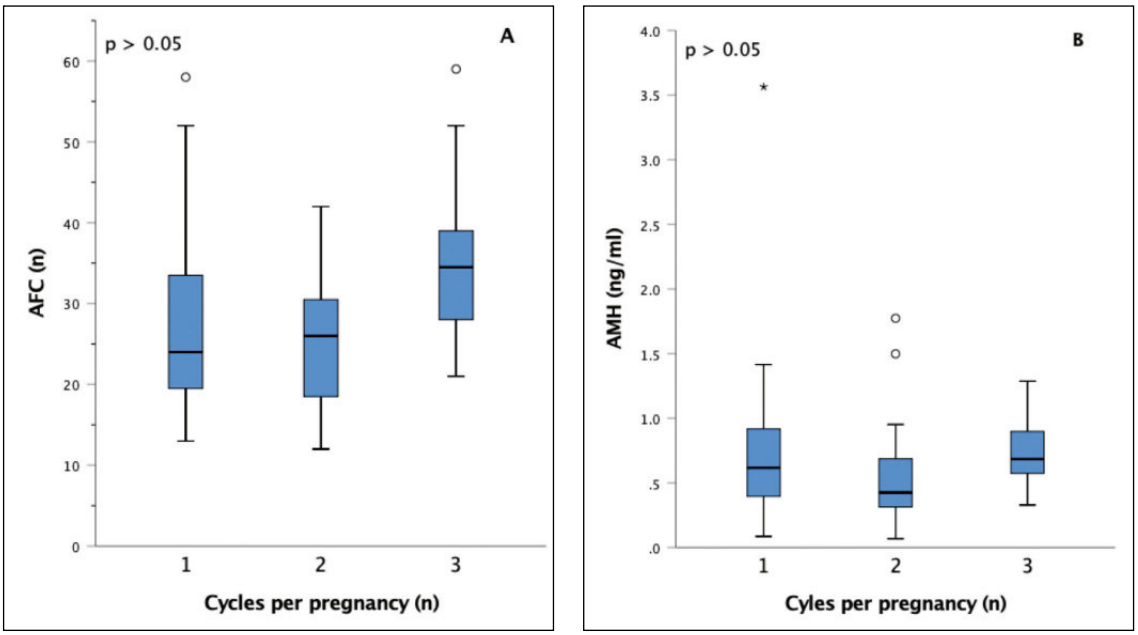


**Figure 10**: Antral follicle count (AFC; Fig. A) and anti-Müllerian hormone concentration (AMH; Fig. B) in mares which got pregnant within one (n = 53), two (n = 27) or three (n = 11) cycles during the breeding season. The boxplots present median values, 25% and 75% quartiles, 1.5 interquartile range (IQR) and outliers, indicated by individual dots and asterisks (representing extreme values).

sponse and fertility in cows.[16,17,29,37] In women, serum AMH is often used to predict success in assisted reproduction techniques[40–42] However, a study in mares by Hanlon et al. (2018)[34] couldn't demonstrate an association between fertility and AMH. In addition, studies in women reported that in patients with extreme low AMH range and especially in young patients, this marker is not able to predict pregnancy after IVF treatments.[35] Another study showed that even older women with low AMH values did not have a significantly different predicted probability of conceiving naturally.[33] Hence, in both mares and women, the ability of AMH to predict fertility outcomes remains controversial.

The reproductive status of the mares was neither related with the AFC nor with AMH. This findings are consistent to those of another study, which also measured similar AMH concentrations in lactating mares with a foal aside and nonlactating mares.[63] A lactating mare is a female with a proven fertility. Therefore, following the hypothesis that a high fertility is linked with higher AFC and AMH, a lactating mare is expected to show higher AFC and AMH compared to barren mares having difficulties to become pregnant. The lack of associations between reproductive status, AFC and AMH, respectively, leads to the assumption that fertility in mares might be more affected by uterine than by ovarian disturbances. Interestingly, the last-mentioned study showed that a delayed uterine clearance after insemination was associated with lower AMH concentrations in blood compared to mares with a physiological uterine clearance.[63] However, only a small number of animals was included in that study and the presence of inflammation wasn't assessed cytologically. Therefore, the described association could not be explained by the authors. In the current study, mares with delayed uterine clearance didn't have reduced AFC and AMH concentrations, nor reduced pregnancy rates compared to the other mares.

Despite the limitations of this field study – such as the unequal distribution of mares across age groups and the use of numerous different stallions – clinically relevant results were found. There are high variabilities of both AMH and AFC, and these parameters are related with each other, showing a stronger relationship in older mares. Moreover, based on our findings, AFC and AMH do not seem to be useful predictors of fertility in mares.

## Relation entre le compte des follicules antraux, le taux sérique d'hormone antimullérienne et la fertilité chez les juments

L'hormone antimullérienne (AMH) agit en tant régulateur de la folliculogénèse en inhibant le recrutement des follicules primordiaux et la croissance folliculaire. Le compte des follicules antraux («antral follicle count», AFC) est fortement corrélé avec le taux sérique d'AMH et les deux semblent être liés à l'état de la réserve ovarienne dans plusieurs espèces.
Il existe peu de données sur l'AMH et l'AFC chez les juments, particulièrement en rapport avec la fertilité. L'objectif de cette étude était donc d'examiner la relation entre le compte de follicules antraux, le taux sérique d'AMH et la fécondité chez les juments. Cent vingt-sept juments ont été inclues dans l'étude et groupées selon leur état reproducteur. Les taux sériques d'AMH et l'AFC ont été déterminés pendant l'oestrus avant et après l'ovulation. Les juments ont été inséminées artificiellement et le diagnostic de gestation réalisé 14 à 18 jours plus tard. Une grande variabilité interindividuelle de l'AFC et l'AMH et une corrélation positive entre l'AMH et l'AFC pour les follicules de diamètre ≤ 30 mm ont été observées, cette dernière étant plus forte chez les juments âgées de plus de 18 ans. L'AFC après ovulation était supérieur à l'AFC avant ovulation, et une forte corrélation entre les deux mesures a été

## Relazione tra la conta dei follicoli antrali, la concentrazione sierica di ormone antimulleriano e la fertilità nelle cavalle

L'ormone antimulleriano (AMH) funge da regolatore della follicologenesi inibendo il reclutamento e lo sviluppo follicolare iniziale. La conta dei follicoli antrali («antral follicle count», AFC) è altamente correlata alla concentrazione sierica di AMH ed entrambi sembrano avere valore predittivo sulla riserva ovarica in diverse specie. Pochi dati sono disponibili sull'AMH e la AFC nelle cavalle, specialmente in relazione alla fertilità. Lo scopo di questo studio era quindi quello di indagare sull'interrelazione tra la conta dei follicoli antrali, i livelli sierici di AMH e la fertilità nelle cavalle. Centoventisette cavalle sono state arruolate nello studio e divise in gruppi in base al loro stato riproduttivo. Le concentrazioni sieriche di AMH e l'AFC sono state determinate durante l'estro prima e dopo l'ovulazione. Le cavalle sono state inseminate artificialmente e la diagnosi di gravidanza è stata effettuata 14-18 giorni più tardi. Sono state osservate un'alta variabilità interindividuale ed una correlazione positiva tra l'AMH e la AFC per i follicoli con diametro ≤ 30 mm, la quale era più forte nelle cavalle di età superiore a 18 anni. La AFC dopo l'ovulazione era più alta della AFC prima dell'ovulazione, ed è stata riscontrata un'elevata correlazione tra i due conteggi. I livelli sierici di AMH non erano in relazione

constatée. Aucun lien entre les taux sériques d'AMH, l'état reproducteur, l'âge, le nombre de cycles œstraux par gestation et le taux de gestation n'a été observé. Hormis un AFC supérieur chez les juments d'âge moyen (9-18 ans) comparé aux juments plus jeunes (< 9 ans), aucune association entre l'AFC, l'âge, l'état reproducteur et la fertilité des juments n'a été trouvée. Cette étude confirme la corrélation entre l'AFC et l'AMH, ainsi qu'un haut degré de reproductibilité du compte des follicules antraux. Toutefois, sur la base de nos observations, ni l'AFC ni l'AMH semblent être de bons prédicteurs de la fertilité.

**Mots clés:** AMH, équin, compte folliculaire, ovaire, état reproducteur, échographie

con lo stato riproduttivo delle cavalle, né con la loro età, il numero di cicli estrali per gravidanza e tasso di gravidanza. Non sono state identificate associazioni tra AFC, età, stato riproduttivo e fertilità delle cavalle, ad eccezione di una AFC maggiore nelle cavalle di media età (9-18 anni) rispetto a quelle più giovani (< 9 anni). Questo studio conferma la correlazione tra AFC e AMH, nonché l'altro grado di riproducibilità della conta dei follicoli antrali. Tuttavia, sulla base dei nostri risultati, né la AFC né i livelli sierici di AMH sembrano essere predittivi della fertilità.

**Parole chiave:** AMH, equini, conta dei follicoli, ovaia, stato riproduttivo, ecografia

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